

Docket No.: 216984US0RE

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :  
Masaru ISHIHARA et al. : ATTN: APPLICATION DIVISION  
REISSUE OF: 6,060,289 :  
FILED: HERewith :  
FOR: MODIFIED BACTERIAL :  
CELLULOSE :

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS  
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Please amend the specification as follows.

Please replace the paragraph at column 1, lines 49-64, with the following:

--Thus, the present invention provides, bacterial cellulose comprising ribbon-shaped microfibrils having a thickness of 1 to 9 nm and a width of 160 to 1000 nm, a method of producing bacterial cellulose which comprises culturing cellulose-producing bacteria which produce the bacterial cellulose extracellularly in a culture medium containing a cell division inhibitor, and recovering the bacterial cellulose produced in the culture medium, and further the present invention provides bacterial cellulose comprising ribbon-shaped microfibrils

having a thickness of 1 to 9 nm and a width of 50 to 70 nm, and a method of producing bacterial cellulose which comprises culturing cellulose-producing bacteria which produce the bacterial cellulose extracellularly in a culture medium containing an organic reducing agent, and recovering the bacterial cellulose produced in the culture medium.--

Please replace the paragraph at column 2, line 63 to column 3, line 6 with the following:

--The bacterial cellulose of the invention comprises ribbon-shaped microfibrils having a minor axis of 1 to 9 nm and a major axis of 160 to 1000 nm or 50 to 70 nm. The inventors cultured cellulose producing bacteria (*Acetobacter pasteurianus* FERM BP-4176) in a culture medium without containing cell division inhibitor and organic reducing agent, and the size of the microfibrils of the bacterial cellulose was measured. As a result, the microfibril had a minor axis of 1 to 9 nm and a major axis of 80 to 150 nm. Accordingly, the bacterial cellulose of the invention is clearly different from conventional bacterial cellulose.--

Please replace the paragraph at column 3, lines 7-13, with the following:

--The minor axis of microfibrils is 1 to 9 nm irrespective of the bacterial cellulose of the invention obtained by culturing in a culture medium containing a cell division inhibitor or an organic reducing agent or conventional bacterial cellulose obtained by culturing in a culture medium not containing cell division inhibitor and organic reducing agent.--

Please replace the paragraph at column 3, lines 14-28, with the following:

--On the other hand, the major axis of the microfibrils of the bacterial cellulose obtained by culturing in a culture medium containing a cell division inhibitor is, in general, 160 to 700 nm, particularly 170 to 600 nm, occasionally longer size, e.g. 1000 nm. That is, the major axis is considerably greater compared with conventional major axis of 80 to 150 nm. When a culture medium contains a cell division inhibitor, cellulose-producing bacteria

are lengthened, and it is observed that a plurality of single chains are adhered to each other to form a bundle. The bundle can be deemed single chain, and accordingly, the major axis becomes considerably longer than conventional one. The ratio of major axis minor axis is about 28:1.0 to 1000:1, particularly, 28:1.0 to 280:1

Please replace the paragraph at column 3, lines 29-35, with the following:

--In the case of the bacterial cellulose obtained by culturing in a culture medium containing an organic reducing agent, the major axis of the microfibrils is, in general, 50 to 70 nm, and it is difficult to discriminate the major axis and the minor axis. It is considered to be caused by shortening of bacterial cell.--

Please replace the paragraph at column 7, lines 6-16, with the following:

--The ribbon-shaped microfibrils produced in NA-added media were observed by the electron microscope and the atomic force microscope, and found that the major axes (width) was great, e.g. 170 nm, 340 nm, 430 nm, 590 nm, etc. , but the minor axes (thickness) were in the range of 1 to 9 nm, e.g., 2.5 nm, 3 nm, 6 nm, 9 nm etc. On the other hand, the ribbon-shaped microfibrils produced in no NA added medium had a major axis (width) of 82 nm, 107 nm, etc and a minor axis (thickness) in the range of 1 to 9 nm, and significant variation was not observed compared with NA added medium concerning the minor axis.--

Please replace the paragraph at column 8, lines 38-48, with the following:

--The CP ribbon-shaped microfibrils produced in NA-added media were observed by the electron microscope and the atomic force microscope, and found that the major axes (width) was great, e.g. 160 nm, 330 nm, 450 nm, 570 nm, 690 nm, etc., but the minor axes (thickness) were in the range of 1 to 9 nm. On the other hand, the ribbon-shaped microfibrils produced in no CP added medium had a major axis (width) of 82 nm, 107 nm, etc and a

minor axis (thickness) in the range of 1 to 9 nm, and significant variation was not observed compared with CP added medium concerning the minor axis.--

Please replace the paragraph at column 9, lines 39-50, with the following:

--The DTT ribbon-shaped microfibrils produced in NA-added media were observed by the electron microscope and the atomic force microscope, and found that the major axes (width) was small, e.g. 56 nm, 57 nm, 70 nm, etc., but the minor axes (thickness) were in the range of 1 to 9 nm. On the other hand, the ribbon-shaped microfibrils produced in no DTT added medium had a major axis (width) of 82 nm, 107 nm, etc and a minor axis (thickness) in the range of 1 to 9 nm, and significant variation was not observed compared with DTT added medium concerning the minor axis.--

Please replace the Abstract with the substitute Abstract attached hereto.

#### IN THE CLAIMS

Please cancel Claims 4, 5 and 6.

Please amend the claims as follows. For the Examiner's convenience, all of the pending claims are reproduced below.

--1. (Amended) A bacterial cellulose comprising microfibrils having a thickness of 1 to 9 nm and a width of 250 to 1000 nm.

2. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have a width of 250 to 700 nm.

3. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have a width of 250 to 600 nm.

7. The bacterial cellulose of claim 1, wherein the microfibrils are ribbon-shaped.

8. A method of producing the bacterial cellulose of claim 1, which comprises culturing cellulose-producing bacteria which produce the bacterial cellulose extracellularly in a culture medium containing a cell division inhibitor, and recovering the bacterial cellulose produced in the culture medium.

9. The method of claim 8, wherein the cell division inhibitor is selected from the group consisting of chloramphenicol, a protein synthesis inhibitor, an organic compound having  $\beta$ -lactamase inhibiting ability, nalidixic acid, promidic acid, pipemidic acid, oxolinaic acid, ofloxacin and enoxacin.

10. The method of claim 9, wherein the protein synthesis inhibitor is selected from the group consisting of tetracycline, puromycin and erythromycin.

11. The method of claim 9, wherein the organic compound having  $\beta$ -lactamase inhibiting ability is thienamycin.

12. The method of claim 8, wherein the concentration of the cell division inhibitor in the culture medium is 0.01 to 5 mM.

13. The method of claim 8, wherein the bacteria are *Acetobacter*.

14. The method of claim 8, wherein the bacteria are *Acetobacter pasteurianus* FERM BP-4176.

15. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have a width of 430 to 1000 nm.

16. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have a width of 590 to 1000 nm.

17. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have a Young's modulus of about 13 to 20 GPa.

18. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have a Young's modulus of about 16 to 20 Gpa.

19. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have a width of 340 to 1000 nm.

20. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have a width of 340 to 700 nm.

21. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have a width of 340 to 600 nm.--

Please add the following claims.

--22. (New) The bacterial cellulose of claim 1, wherein the microfibrils have a thickness of 2.5, 3, 6, or 9 nm.

23. (New) The bacterial cellulose of claim 1, wherein the ratio of the major axis to the minor axis of the microfibrils is about 28:1.0 to 1000:1.0

24. (New) The bacterial cellulose of claim 1, wherein the ratio of the major axis to the minor axis of the microfibrils is about 28:1.0 to 280:1.0.

25. (New) A bacterial cellulose produced by *Acetobacter pasteurianus* FERM BP-4176 which comprises microfibrils having a thickness of 1 to 9 nm and a width of 250 to 1000 nm.

26. (New) The bacterial cellulose of claim 25, wherein the microfibrils have a width of 250 to 700 nm.

27. (New) The bacterial cellulose of claim 25, wherein the microfibrils have a width of 250 to 600 nm.

28. (New) The bacterial cellulose of claim 25, wherein the microfibrils have a width of 430 to 1000 nm.

29. (New) The bacterial cellulose of claim 25, wherein the microfibrils have a width of 590 to 1000 nm.

30. (New) The bacterial cellulose of claim 25, wherein the microfibrils have a width of 340 to 1000 nm.

31. (New) The bacterial cellulose of claim 25, wherein the microfibrils have a width of 340 to 700 nm.

32. (New) The bacterial cellulose of claim 25, wherein the microfibrils have a width of 340 to 600 nm.

33. (New) The bacterial cellulose of claim 25, wherein the microfibrils have a Young's modulus of about 13 to 20 GPa.

34. (New) The bacterial cellulose of claim 25, wherein the microfibrils have a Young's modulus of about 16 to 20 GPa.

35. (New) The bacterial cellulose of claim 25, wherein the ratio of the major axis to the minor axis of the microfibrils is about 28:1.0 to 1000:1.0.

36. (New) The bacterial cellulose of claim 25, wherein the ratio of the major axis to the minor axis of the microfibrils is about 28:1.0 to 280:1.0.

37. (New) The bacterial cellulose of claim 25, wherein the microfibrils are ribbon-shaped.

38. (New) A method of producing the bacterial cellulose of claim 25, which comprises culturing cellulose-producing bacteria which produce the bacterial cellulose extracellularly in a culture medium containing a cell division inhibitor, and recovering the bacterial cellulose produced in the culture medium.

39. (New) The method of claim 38, wherein the cell division inhibitor is selected from the group consisting of chloramphenicol, a protein synthesis inhibitor, an organic

compound having  $\beta$ -lactamase inhibiting ability, nalidixic acid, promidic acid, pipemidic acid, oxolinaic acid, ofloxacin and enoxacin.

40. (New) The method of claim 39, wherein the protein synthesis inhibitor is selected from the group consisting of tetracycline, puromycin and erythromycin.

41. (New) The method of claim 39, wherein the organic compound having  $\beta$ -lactamase inhibiting ability is thienamycin.

42. (New) The method of claim 38, wherein the concentration of the cell division inhibitor in the culture medium is 0.01 to 5 mM.

43. (New) The method of claim 38, wherein the bacteria are *Acetobacter*.

44. (New) The method of claim 38, wherein the bacteria are *Acetobacter pasteurianus* FERM BP-4176.--

#### REMARKS

This is a reissue application of U.S. patent No. 6,060,289, issued May 9, 2000.

Original patent Claims 1-3 and 7-21 are active in this application. Claims 22-44 have been added in the amendment submitted above.

The present reissue has been filed, *inter alia*, to correct an error in the reported thickness of the microfibrils and the ratio of the major axis to the minor axis of the microfibrils of the inventive bacterial cellulose.

Claim 1 as amended and newly added Claim 25 each specify that the microfibrils have a thickness of 1 to 9 nm. Newly added Claim 22 specifies that the microfibrils have a width of 2.5, 3, 6, or 9 nm.

As described in the executed Declaration Under 37 C.F.R. §1.175 from the inventors in the present application, Masaru Ishihara and Shigeru Yamanaka, submitted herewith, the



original value of the thickness and the and the ratio of the major axis to the minor axis of the microfibrils set forth in the application as originally filed was not correct. As explained in the Declaration at pages 2-3, the thickness of the microfibrils in the Examples was correctly determined to be 1 to 9 nm, e.g., 2.5, 3, 6, 9 nm, etc. In addition, as a result of the change in the thickness of the microfibrils described above, the ratio of the major axis/minor axis is changed as well. The lower limit of this ratio is  $250\text{ nm}:9\text{ nm} = 28:1.0$ , and the upper limit is  $1000\text{ nm}:1\text{ nm} = 1000:1.0$ , and with respect to the particular range, the lower limit is  $250\text{ nm}:9\text{ nm} = 28:1.0$  and the upper limit is  $700\text{ nm}:2.5\text{ nm} = 280:1.0$ . See the Declaration at page 3.

Since the thickness and the the ratio of the major axis to the minor axis of the bacterial cellulose microfibrils produced in the Examples of the present application are an inherent property of the material, amending the specification to correct the values of the thickness produced therein is not new matter. See *In re Nathan*, 140 USPQ 601 (CCPA 1964) and *In re Magerlein*, 145 USPQ 683 (CCPA 1965), copies of which are submitted herewith.

The specification has also been amended to correct the error in the description of the minor axis, i.e., the thickness, of the bacterial cellulose microfibrils produced in the Examples of the present application, and the corresponding description of the same in the text of the specification preceding the Examples.

Claims 2-3 and 15-21 have been amended for clarity. In addition, the recitation of "about" in reference to the Young's modulus recited in Claims 17 and 18 is supported by the specification at column 3, lines 36-46.

Newly added Claims 23 and 24 are supported by the specification at column 3, lines 26-27. Newly added Claims 25-44 are supported by the specification at column 1, line 35 to column 10, line 42.

No new matter is believed to have been added to this application by these amendments.

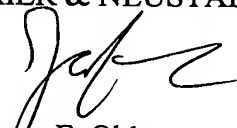
Applicants submit herewith an executed Declaration Under 37 C.F.R. §1.175.

In addition, pursuant to 37 C.F.R. §1.178(a), Applicants offer to surrender the original patent, i.e., U.S. patent No. 6,060,289, issued May 9, 2000.

Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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### IN THE SPECIFICATION

Please amend the specification as follows.

Please replace the paragraph at column 1, lines 49-64, with the following:

--Thus, the present invention provides, bacterial cellulose comprising ribbon-shaped microfibrils having a thickness of 1 to 9 [10 to 100] nm and a width of 160 to 1000 nm, a method of producing bacterial cellulose which comprises culturing cellulose-producing bacteria which produce the bacterial cellulose extracellularly in a culture medium containing a cell division inhibitor, and recovering the bacterial cellulose produced in the culture medium, and further the present invention provides bacterial cellulose comprising ribbon-shaped microfibrils having a thickness of 1 to 9 [10 to 100] nm and a width of 50 to 70 nm, and a method of producing bacterial cellulose which comprises culturing cellulose-producing bacteria which produce the bacterial cellulose extracellularly in a culture medium containing an organic reducing agent, and recovering the bacterial cellulose produced in the culture medium.--

Please replace the paragraph at column 2, line 63 to column 3, line 6 with the following:

--The bacterial cellulose of the invention comprises ribbon-shaped microfibrils having a minor axis of 1 to 9 [10 to 1000] nm and a major axis of 160 to 1000 nm or 50 to 70 nm. The inventors cultured cellulose producing bacteria (*Acetobacter pasteurianus* FERM BP-4176) in a culture medium without containing cell division inhibitor and organic reducing

agent, and the size of the microfibrils of the bacterial cellulose was measured. As a result, the microfibril had a minor axis of 1 to 9 [10 to 100] nm and a major axis of 80 to 150 nm. Accordingly, the bacterial cellulose of the invention is clearly different from conventional bacterial cellulose.--

Please replace the paragraph at column 3, lines 7-13, with the following:

--The minor axis of microfibrils is 1 to 9 nm [, in general, 55 to 95 nm, occasionally smaller size, e.g., 25 nm,] irrespective of the bacterial cellulose of the invention obtained by culturing in a culture medium containing a cell division inhibitor or an organic reducing agent or conventional bacterial cellulose obtained by culturing in a culture medium not containing cell division inhibitor and organic reducing agent.--

Please replace the paragraph at column 3, lines 14-28, with the following:

--On the other hand, the major axis of the microfibrils of the bacterial cellulose obtained by culturing in a culture medium containing a cell division inhibitor is, in general, 160 to 700 nm, particularly 170 to 600 nm, occasionally longer size, e.g. 1000 nm. That is, the major axis is considerably greater compared with conventional major axis of 80 to 150 nm. When a culture medium contains a cell division inhibitor, cellulose-producing bacteria are lengthened, and it is observed that a plurality of single chains are adhered to each other to form a bundle. The bundle can be deemed single chain, and accordingly, the major axis becomes considerably longer than conventional one. The ratio of major axis/minor axis is about 28:1.0 to 1000:1 [2.8:1.0 to 8.1:1.0], particularly, 28:1.0 to 280:1 [3.0:1.0 to 6.0:1.0. In the case of conventional microfibrils, the ratio of major axis/minor axis is 1.6:1.0 to 2.7:1.0.]--

Please replace the paragraph at column 3, lines 29-35, with the following:

--In the case of the bacterial cellulose obtained by culturing in a culture medium containing an organic reducing agent, the major axis of the microfibrils is, in general, 50 to 70 nm, and it is difficult to discriminate the major axis and the minor axis. It is considered to be caused by shortening of bacterial cell. [The ratio of major axis: minor axis is about 0.9:1.0 to 1.5:1.0, particularly, 1.2:1.0 to 1.5:1.0.]--

Please replace the paragraph at column 7, lines 6-16, with the following:

--The ribbon-shaped microfibrils produced in NA-added media were observed by the electron microscope and the atomic force microscope, and found that the major axes (width) was great, e.g. 170 nm, 340 nm, 430 nm, 590 nm, etc. , but the minor axes (thickness) were in the range of 1 to 9 nm, e.g., 2.5 nm, 3 nm, 6 nm, 9 nm [10 to 100 nm, e.g., 25, 30, 60, 90 nm] etc. On the other hand, the ribbon-shaped microfibrils produced in no NA added medium had a major axis (width) of 82 nm, 107 nm, etc and a minor axis (thickness) in the range of 1 to 9 [10 to 100] nm, and significant variation was not observed compared with NA added medium concerning the minor axis.--

Please replace the paragraph at column 8, lines 38-48, with the following:

--The CP ribbon-shaped microfibrils produced in NA-added media were observed by the electron microscope and the atomic force microscope, and found that the major axes (width) was great, e.g. 160 nm, 330 nm, 450 nm, 570 nm, 690 nm, etc., but the minor axes (thickness) were in the range of 1 to 9 [10 to 100] nm. On the other hand, the ribbon-shaped microfibrils produced in no CP added medium had a major axis (width) of 82 nm, 107 nm, etc and a minor axis (thickness) in the range of 1 to 9 nm, and significant variation was not observed compared with CP added medium concerning the minor axis.--

Please replace the paragraph at column 9, lines 39-50, with the following:

--The DTT ribbon-shaped microfibrils produced in NA-added media were observed by the electron microscope and the atomic force microscope, and found that the major axes (width) was small, e.g. 56 nm, 57 nm, 70 nm, etc., but the minor axes (thickness) were in the range of 1 to 9 [10 to 100] nm. On the other hand, the ribbon-shaped microfibrils produced in no DTT added medium had a major axis (width) of 82 nm, 107 nm, etc and a minor axis (thickness) in the range of 1 to 9 [10 to 100] nm, and significant variation was not observed compared with DTT added medium concerning the minor axis.--

Please replace the Abstract with the substitute Abstract attached hereto.

#### IN THE CLAIMS

Please cancel Claims 4, 5 and 6.

Please amend the claims as follows. For the Examiner's convenience, all of the pending claims are reproduced below.

--1. (Amended) A bacterial cellulose comprising microfibrils having a thickness of 1 to 9 [10 to 100] nm and a width of 250 to 1000 nm.

2. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have [which has] a width of 250 to 700 nm.

3. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have [which has] a width of 250 to 600 nm.

7. The bacterial cellulose of claim 1, wherein the microfibrils are ribbon-shaped.

8. A method of producing the bacterial cellulose of claim 1, which comprises culturing cellulose-producing bacteria which produce the bacterial cellulose extracellularly in a culture medium containing a cell division inhibitor, and recovering the bacterial cellulose produced in the culture medium.

9. The method of claim 8, wherein the cell division inhibitor is selected from the group consisting of chloramphenicol, a protein synthesis inhibitor, an organic compound having  $\beta$ -lactamase inhibiting ability, nalidixic acid, promidic acid, pipemidic acid, oxolinic acid, ofloxacin and enoxacin.
10. The method of claim 9, wherein the protein synthesis inhibitor is selected from the group consisting of tetracycline, puromycin and erythromycin.
11. The method of claim 9, wherein the organic compound having  $\beta$ -lactamase inhibiting ability is thienamycin.
12. The method of claim 8, wherein the concentration of the cell division inhibitor in the culture medium is 0.01 to 5 mM.
13. The method of claim 8, wherein the bacteria are Acetobacter.
14. The method of claim 8, wherein the bacteria are Acetobacter pasteurianus FERM BP-4176.
15. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have [which has] a width of 430 to 1000 nm.
16. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have [which has] a width of 590 to 1000 nm.
17. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have [which has] a Young's modulus of about 13 to 20 GPa.
18. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have [which has] a Young's modulus of about 16 to 20 GPa.
19. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have [which has] a width of 340 to 1000 nm.

20. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have  
[which has] a width of 340 to 700 nm.

21. (Amended) The bacterial cellulose of claim 1, wherein the microfibrils have  
[which has] a width of 340 to 600 nm.--

Please add the following claims.

--22. (New)

23. (New)

24. (New)

25. (New)

26. (New)

27. (New)

28. (New)

29. (New)

30. (New)

31. (New)

32. (New)

33. (New)

34. (New)

35. (New)

36. (New)

37. (New)

38. (New)

39. (New)

40. (New)

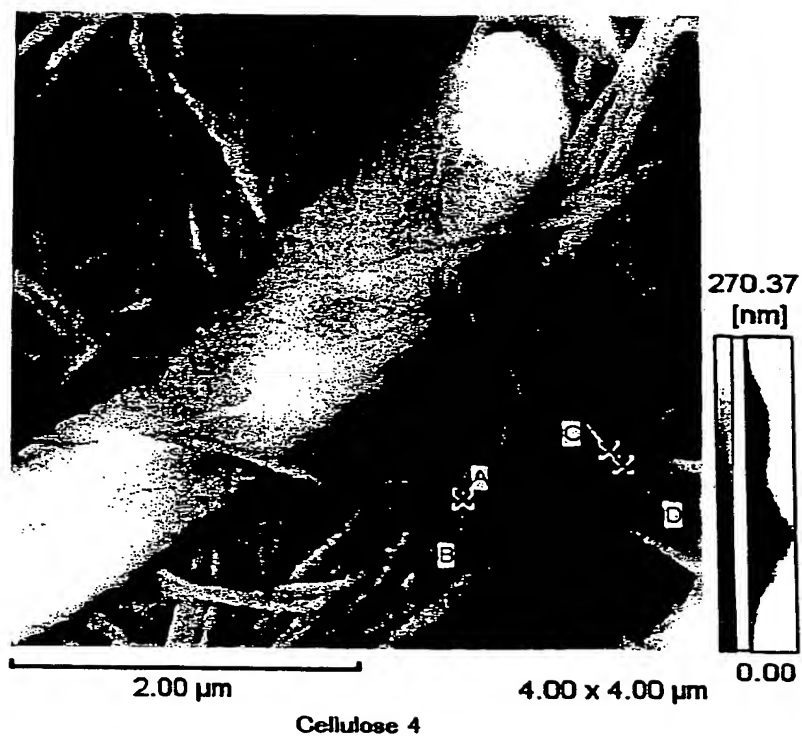


41. (New)

42. (New)

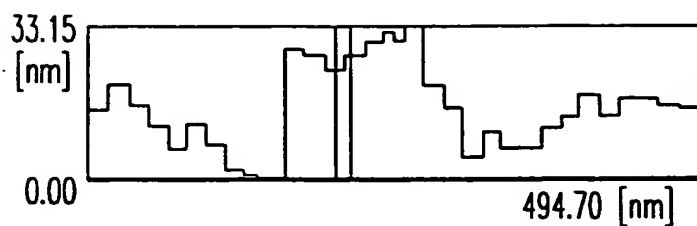
43. (New)

44. (New)--



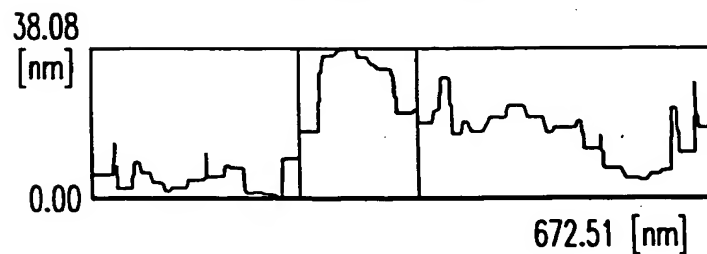
*FIG. 1*

2020.01.10 14:00:00 0.00 0.00 0.00



	DISTANCE [nm]	DIFFERENCE IN HEIGHT [nm]	ANGLE [°]
—	11.02	3.23	16.36

**FIG. 2**



	DISTANCE [nm]	DIFFERENCE IN HEIGHT [nm]	ANGLE [°]
—	124.32	2.23	1.03

**FIG. 3**

### ABSTRACT

This invention provides a bacterial cellulose comprising ribbon-shaped microfibrils having a thickness of 1 to 9 nm and a width of 160 to 1000 nm or a bacterial cellulose comprising ribbon-shaped microfibrils having a thickness of 1 to 9 nm and a width of 50 to 70 nm. The former bacterial cellulose can be produced by culturing cellulose-producing bacteria in a culture medium containing a cell division inhibitor, and the latter can be produced by culturing the bacterium in a culture medium containing an organic reducing agent. The bacterial cellulose is modified from conventional bacterial cellulose in the major axis, and is improved in Young's modulus, etc.

### Example 1

The culture medium used was composed of 50.0 g/l sucrose, 5.0 g/l "Total Amino Acid" (Ajinomoto Co., Inc.), 0.2 g/l phytic acid, 2.4 g/l magnesium sulfate and 1.0 g/l ammonium sulfate (pH 5.0).

Seed culture was carried out by placing 20 ml of the above culture medium in a 100 ml flask with baffle, inoculating Acetobacter pasteurianus FERM BP-4176, and then culturing at 25 °C for 3 days with stirring at 200 rpm. The culture medium was crushed by a blender, and added to a main culture medium having the above composition in a concentration of 2 % seed culture.

The main culture was carried out by static culture at 25 °C. During the culture, culture solution and bacterial cellulose were withdrawn, and the morphology of bacteria was observed by an optical microscope, an electron microscope and an atomic force microscope.

Six main culture media were used, and nalidixic acid (NA) was added thereto in a concentration of 0.01 mM, 0.05 mM, 0.1 mM, 0.2 mM or 1.0 mM except one medium to which NA was not added.

As a result, production of bacterial cellulose was inhibited with increasing the NA concentration. For example, the shape of the bacterium after cultured in the medium containing 0.1 mM NA and that cultured in the medium not

containing NA for 2 days were compared by taking each an optical microscope photograph ( $\times 1000$ ). As a result, in the case of 0.1 mM NA, the shape of bacterium was varied and lengthened 2 to 4 times compared with no addition of NA.

The ribbon-shaped microfibrils produced in NA-added media were observed by the atomic force microscope, and found that the major axes (width) was great, e.g. 340 nm, 430 nm, 590 nm, etc., but the minor axes (thickness) were in the range of 1 to 9 nm, e.g. 2.5 nm, 3 nm, 6 nm, 9 nm etc. On the other hand, the ribbon-shaped microfibrils produced in no NA added medium had a major axis (width) of 82 nm, 107 nm, etc and a minor axis (thickness) in the range of 1 to 9 nm, and significant variation was not observed compared with NA added medium concerning the minor axis.

## Example 2

Acetobacter pasteurianus FERM BP-4176 was cultured in static culture, and the culture solution and bacterial cellulose were withdrawn, and the shape of bacteria was observed by the optical microscope, the electron microscope and the atomic force microscope, similar to Example 1, except that chloramphenicol was used instead of nalidixic acid.

That is, six main culture media having the aforementioned composition were used, and chloramphenicol (CP) was added thereto in a concentration of 0.1mM, 0.2mM, 0.3mM, 0.5mM or 1.0mM except one medium to which CP was not added.

As a result, the length of the cellulose-producing bacterium increased with increasing the CP concentration up to 8 to 12 times as long as the bacteria cultured in no CP medium.

The CP ribbon-shaped microfibrils produced in CP-added media were observed by the atomic force microscope, and found that the major axes (width) was great, e.g. 330 nm, 450 nm, 570 nm, 690 nm, etc., but the minor axes (thickness) were in the range of 1 to 9 nm. On the other hand, the ribbon-shaped microfibrils produced in no CP added medium had a major axis (width) of 82 nm, 107 nm, etc and a minor axis (thickness) in the range of 1 to 9 nm, and significant variation was not observed compared with CP added medium concerning the minor axis.

#### Example 4

Acetobacter pasteurianus FERM BP-4176 was cultured in agitation culture at 180 rpm instead of static culture, and the culture solution and bacterial cellulose were withdrawn, and the shape of bacteria was observed by the optical microscope, the electron microscope and the atomic force microscope, similar to Example 1.

That is, four main culture media having the aforementioned composition were used, and nalidixic acid (NA) was added thereto in a concentration of 0.10 mM, or 0.20 mM, except one medium to which NA was not added.

As a result, the length of the cellulose-producing bacteria increased. The ribbon-shaped microfibrils produced in NA-added media were observed by the atomic force microscope, and found that the major axes (width) was great, e.g. 250nm, 350nm, etc., but variation in the minor axes was not observed.